DESTRUCTION OF TRANSFUSED BLOOD IN NORMAL SUBJECTS AND IN PERNICIOUS ANEMIA PATIENTS

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STUDY OF TRANSFUSED BLOOD.

I. The Periodicity in Eliminative Activity Shown by the Organism.

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STUDY OF TRANSFUSED BLOOD.

I. THE PERIODICITY IN ELIMINATIVE ACTIVITY SHOWN BY THE ORGANISM.

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It was hoped that some light might be thrown on the length of life of normal blood corpuscles and the mechanism of their removal from the circulation by the study of the elimination of transfused blood, which it is possible to make when the transfused blood is of a group unlike that of the recipient. This study has brought to light two facts, first, that the length of time that transfused blood remains in the circulation varies greatly; and second, that the elimination is not a continuous process but takes place in more or less cyclic crises, so that the responsibility for the disappearance of transfused blood from the circulation seems to rest more heavily on this cyclic activity of the body than upon the condition of the corpuscle.

The probable life of the blood corpuscle was reduced to 10 days or less by calculation based upon bile pigment output, made by Zoja and others, after the time of Ward-Muller, Quinck, von Ott, and Hunter, who from transfusion experiments in dogs concluded that the life of the normal corpuscle was from 14 to 26 days. The experimental results of Todd and White (1909–10) upon cattle, obtained by using an isohemolytic serum to separate out transfused blood, are in agreement with the shorter period. The work of Whipple and Hooper, however, who show that bile pigment is only in part derived from blood pigment, has made the shorter calculations based on bile pigment excretion untenable. The observations of Rous and Turner, who kept rabbit corpuscles *in vitro* for 14 days and then substituted them for a rabbit's own blood without any abnormal results, would also indicate a longer life of the corpuscles, as do my own results (1919, *a*) of the study of transfused blood in man.

As for the mechanism of blood elimination, we must undoubtedly give credit to some extent to phagocytosis, which plays a more or less important part in all

animals and appears, moreover, to be capable of extension under stimulation. Keyes finds in a wide range of animals that the physiologic destruction of the animals' own red blood corpuscles is accomplished by specialized fixed tissue phagocytes, which are confined largely to the liver or to the spleen. In birds, amphibia, and the lower mammals the phagocytic activity is most marked in the liver; in the higher animals it is most marked in the spleen. Rous and Robertson (1917) made quantitative estimates of the presence of phagocytes in the spleen of guinea pigs, cats, dogs, rabbits, *rhesus* monkeys, and man, and found that although in some of these animals phagocytic cells were present in numbers that might account for physiologic blood destruction, in the monkey and man these were few. Muir, in the study of phagocytosis in a rather large series of cases of empyema, smallpox, and pneumonia, found that phagocytosis was negligible in the bone marrow, but that in the spleen phagocytic activity was displayed against the native red blood corpuscles by cells of the splenic pulp and by certain hyaline leucocytes within the pulp. Carey, using intravenous injections of foreign red cells, and Downey, using intravenous injections of dye granules, found that under increased stimulation there is an increase in the number of cells which act as phagocytes. Downey believes that many "connective tissue" cells, may, under stimulation, become phagocytic. Carey finds under repeated stimulation an extension of phagocytic activity from cells of the spleen to those of the liver.

Rous and Robertson (1917) have recently given us knowledge of a second method of blood destruction, that of fragmentation in the blood stream with, it would seem, a filtering out of these fragments by the spleen. This they consider in man to have a greater importance than phagocytosis.

There are certain anomalous findings that suggest the possibility that a specific antibody mechanism is responsible for physiologic blood destruction. Todd and White's (1909–10) isohemolytic serum would not hemolyze ox corpuscles *in vitro* in the presence of ox serum unless guinea pig complement was added, but when injected intravenously into oxen produced massive hemolysis. Todd and White considered that this indicated the presence of some organ in the body which was able to produce the activating effect of guinea pig complement. Rous and Robertson (1918) found in some of their rabbits that had been receiving massive doses of rabbit blood that an isoagglutinin developed which caused agglutination of the corpuscles of the animals at room temperature. This agglutinin tended to appear in those rabbits which in the face of heavy blood injections developed an anemia. Davis and Macgregor both report evidence of erythrophagocytic bodies in meningitic patients, and in paroxysmal hemoglobinuria we have the isohemolysin which only unites with complement at low temperatures. Although one is tempted to speculate upon the possibility that physiologic blood corpuscle destruction is produced by some organ or organs of the body that form an antibody having lytic and opsonic qualities and so promoting fragmentation and phagocytosis, we have no evidence that any such antibody exists.

If we can draw a parallel between the elimination of transfused blood and normal blood destruction, the following study of transfused blood makes, I feel, a contribution to our understanding of blood destruction in that it would seem to connect the process with the activity of the endocrine gland system.

Method.

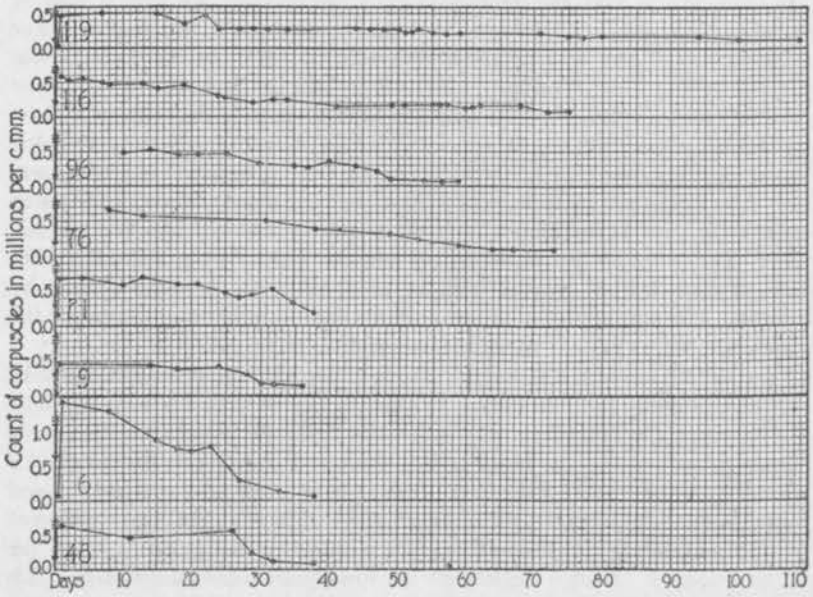
In the following work the elimination of transfused blood has been studied in individuals in Groups I, II, and III receiving Group IV transfusions. The method used was that published by me originally in *The Journal of Experimental Medicine* (1919, *a*), with certain modifications described in the *Medical Clinics of North America* (1919, *b*). It consists, in brief, of diluting the recipient's blood after transfusion with Group IV serum. This agglutinates the native corpuscles, leaving the transfused corpuscles free and capable of being counted in a hemocytometer. The dilution is made in a white blood-counting pipette, Group IV citrated serum being used as the diluent, and the mixture is expelled into Wassermann tubes. The tubes are incubated for 40 minutes with shaking, and allowed to stand at room temperature for from 15 to 30 minutes. A count is made by daylight, or, if it is necessary to use artificial light, care is taken to protect the corpuscles from its effect. All counts are made in duplicate.

Since by the use of this technique it is necessary that the transfused blood be of a different group from the recipient's blood it seemed more desirable, if it were feasible, to use the method of Todd and White (1909–10), which in cattle gives a differentiation between the blood of individuals. Patients who had received a series of from four to five 500 cc. transfusions were tested for hemolysin. Blood plasma from these patients was used both fresh without guinea pig complement and with guinea pig complement against corpuscles of the group to which the plasma belonged. Neither agglutinins nor hemolysins could be detected. This method is not applicable.

In harmony with the current conception of the behavior of native corpuscles, I assumed that transfused blood corpuscles would slowly deteriorate in the circulation and upon reaching a certain degree of degeneration either fragment or be phagocytosed, while their place was taken by new corpuscles. If this should be the case, on the injection of a normal blood into individuals not having blood disease one would expect a certain approximate equality in the length of time that expires before the transfused blood leaves the circulation, and by studying several curves of elimination to the end it seemed probable that a figure could be obtained that would roughly represent the length of life of the newest corpuscles injected.

RESULTS.

In Text-fig. 1 are given the curves of elimination of transfused blood in the eight cases of individuals without blood disease which have been studied to complete elimination or to nearly complete elimination. The time taken for elimination was approximately 100, 72, 49, 63, 38, 30, 34, and 33 days.



TEXT-FIG. 1. Curves showing the variability of the time taken by individuals without blood disease to eliminate Group IV transfused blood. ↧ indicates a Group IV transfusion.

In these data the variation in the time taken to eliminate Group IV transfused blood is so great that no time can be given as the average length of life of the transfused corpuscles. Although slight individual differences in bloods can be noted with respect to resistance to hypertonic salt solution and other hemolysins, it would hardly seem probable that so great a difference in the time of elimination as that between 100 days and 30 days could be attributed to differences in resistance of the transfused bloods, to which dif-

ferences in resistance to hypertonic salt solution might be an index. It will be noted that the longest curve, 100 days (Case 119), was obtained in the case of a man in health, while the elimination time was only 28 to 30 days in the case of a very cachectic cancer patient (Case 48). No generalization, however, can be made from this, as a patient (Case 9) whose blood had returned to normal after hysterectomy eliminated the transfused blood in 28 to 30 days, and a patient who died with long standing infection (Case 95) had shown no definite sign of elimination of the transfused blood after 46 days. The protocols of these individuals are given below.

Case 6.—A woman, aged 35 years; weight 138 pounds. An operation for fibroid of the uterus was performed, followed by two transfusions because of severe hemorrhage.

Case 9.—A woman, aged 33 years, whose normal weight was 100 pounds, was transfused previous to subtotal hysterectomy. The convalescence was satisfactory.

Case 21.—A woman, aged 45 years, weighing 87 pounds, had common duct obstruction, and tertiary syphilis of the liver.

Case 48.—A woman, aged 48 years, weighing 127 pounds, had cancer of the breast with extensive glandular involvement. The menstrual periods had been regular up to February 7, 1919. There was no further menstruation until March 28, when the menstrual flow began and continued until April 23. If the menstrual periods had continued to be regular after February, they should have occurred March 7, April 4, and May 2. On April 5 a radical operation was performed, followed by a transfusion.

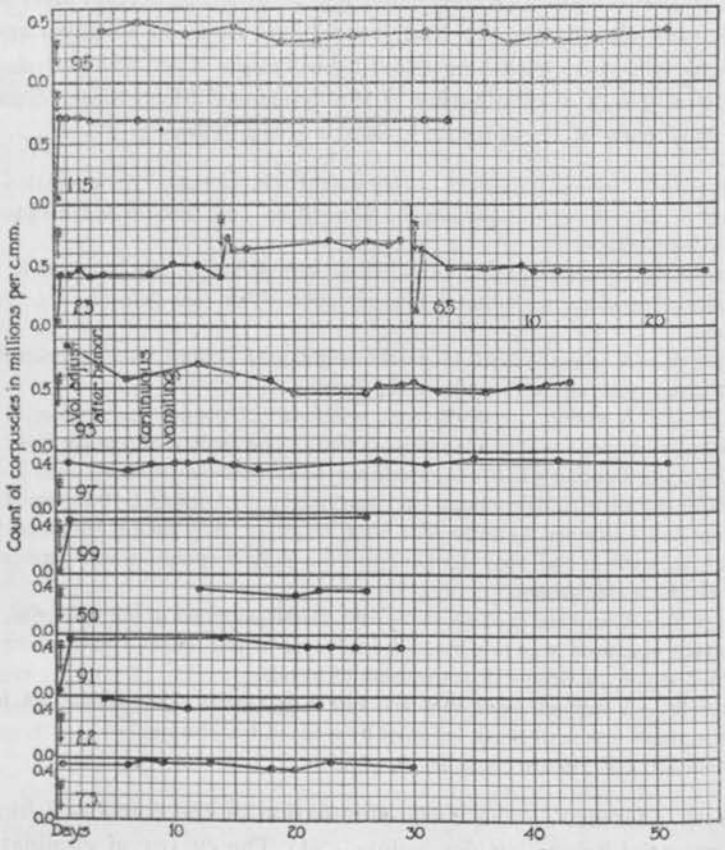
Case 76.—A woman, aged 24 years, had chondrosarcoma of the right ribs. A two-stage operation was performed for removal of the tumor. Each operation was followed by a Group IV transfusion of blood.

Case 96.—A woman, aged 40 years, had a malignant pelvic tumor. A total hysterectomy was performed, followed by a blood transfusion.

Cases 116 and 119 are given below.

With reference to the cyclic elimination of the transfused blood, my material brings out five points. (1) The curves of elimination of Group IV transfused blood do not show a gradual downward curve day by day, which would be expected if it is assumed that blood corpuscles gradually wear out and are replaced. When due allowance has been made for blood volume changes, it will be seen that the count stays on a level for a long time. (2) When the counts have been taken sufficiently close together the curve of elimination

makes a sudden drop to a lower level, at which it again remains for many days. There are longer periods of no elimination and shorter periods of elimination. (3) The nature of these drops is such that they are more likely to be due to an active destruction on the part of the body than to, what would be the other possibility, a coinci-



TEXT-FIG. 2. Curves showing that over a long period of time there is no elimination of transfused blood. ↧ indicates a Group IV transfusion.

dent spontaneous disintegration of the corpuscles. (4) Accompanying these drops in the count of the transfused blood, there is usually an activity of the bone marrow as indicated by a rise in the total blood count. (5) In women these drops are related to the menstrual cycle.

Text-fig. 2 illustrates the fact that over a long period of time there is no elimination of transfused blood. The protocols of these cases follow.

Case 22.—A man, aged 32 years, weighing 103 pounds, had duodenal ulcer and chronic appendicitis. A posterior gastroenterostomy and appendectomy were performed, followed by a transfusion.

Case 23.—A man, aged 57 years, weighing 139½ pounds, had pernicious anemia and was given a series of transfusions.

Case 50.—A man, aged 50 years, a farm laborer of powerful build, received a transfusion after appendectomy and gall bladder operation.

Case 65.—A boy, aged 11 years, weighed 63 pounds. A diagnosis of lymphatic leucemia was made. He was transfused.

Case 73.—A woman, aged 47 years, weighing 120 pounds, had pernicious anemia. She received a series of transfusions.

Case 91.—A woman, aged 53 years, weighing 137 pounds, with pernicious anemia, received a series of transfusions.

Case 93.—A woman, aged 43 years, was given a transfusion because of post-operative hemorrhage after gall bladder operation. On the 8th and 9th days after transfusion subcutaneous injections of saline solution were given because of continuous vomiting.

Case 95.—A man, aged 31 years, had an arteriovenous aneurysm between the popliteal artery and vein, resulting from injury by a bullet. After operation secondary hemorrhage developed, necessitating a transfusion 6 days later. On the 19th day after transfusion the leg was amputated for dry gangrene. On the 55th day following hemorrhage, a pelvic exploration was done. The patient died on the 57th day after transfusion.

Case 97.—A man, aged 42 years, weighing 155 pounds, had pernicious anemia and was given a series of transfusions.

Case 99.—A girl, aged 17 years, weighing 100 pounds, was given a blood transfusion for secondary anemia due to malaria. Following medication there were no more chills.

Case 115.—A woman, aged 33 years, weighing 141 pounds, was given a blood transfusion January 26, 1920, because of secondary anemia. Normal menstruation occurred while the patient was under observation, January 26, February 27, and March 24.

The protocols of cases included in Text-fig. 3 are given below. In Cases 116 and 119 the tabulation of the blood counts, which are omitted in other instances to save the cost of reproduction, are partially given, as the length of time over which the study was made necessitated a reduction in the charts, which leaves them inade-

Length of time after transfusion.	No. of unagglutinated corpuscles per c.mm.
days	
0	33,000
0 (Transfusion with 500 cc. of Group IV citrated blood.)	
0	441,000
1	459,000
7	500,000
15	490,000
19	350,000
22	475,000
24	270,000
27	281,000
29	282,000
31	271,000
34	260,000
44	288,000
46	281,000
48	268,000
50	272,000
51	220,000
52	223,000
53	289,000
55	224,000
56	202,000
58	211,000
60	224,000
70	222,000
74	173,000
76	157,000
79	190,000
93	184,000
100	118,000
109	118,000

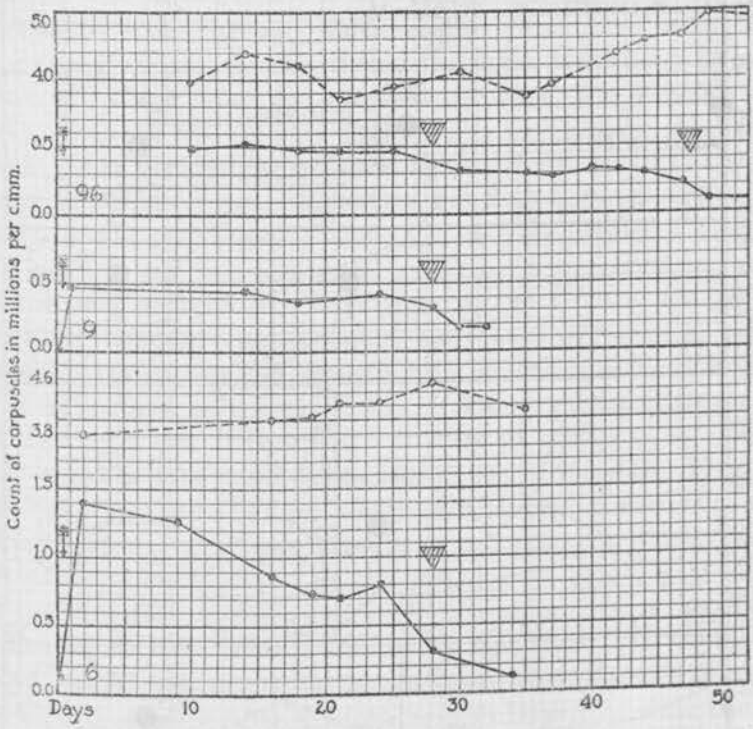
Text-fig. 4 presents the curves of Cases 6, 9, and 96, the protocols of which are given above.

Protocols of cases included in Text-fig. 5 are given below with the exception of Cases 48 and 115 which are presented above.

Case 38.—A woman, aged 32 years, weighing 133 pounds, in good general health had multiple lipomata, which were removed. The following day a transfusion of blood was given because of postoperative hemorrhage. Convalescence was excellent. Menstruation began on the 35th day after transfusion and ended on the 38th day.

Case 47.—A woman, aged 40 years, weighed 105 pounds. A partial thyroidectomy was performed, which was followed by a transfusion. The patient made an uneventful recovery. Menstruation began the 37th day after transfusion.

Case 68.—A woman, aged 36 years, weighing 82 pounds, had pernicious anemia. The patient claimed that her menstrual period was usually 24 days.

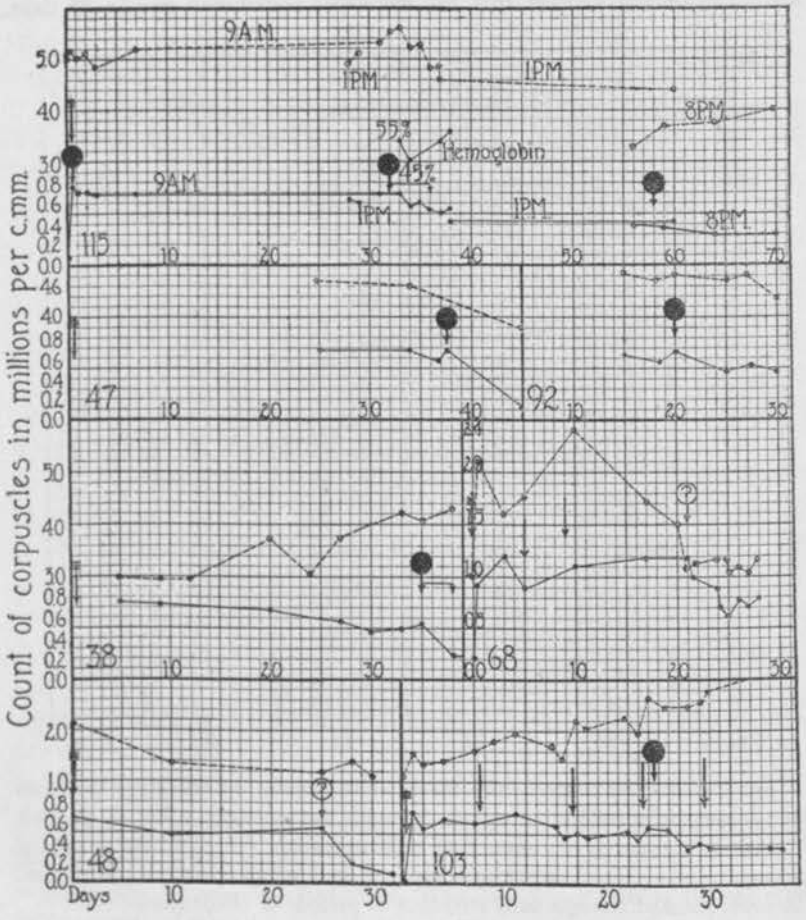


TEXT-FIG. 4. The drops in the curves of elimination of transfused blood of three women who received a transfusion with hysterectomy. The drops took place at approximately the same time after operation. ——— Unagglutinated corpuscles. – – – Total red blood count. ↧ indicates a Group IV transfusion. The cross-hatched triangle calls attention to periods of elimination.

While she was under observation menstruation occurred, beginning 3 days before transfusion, June 24, 1919. Menstruation did not occur July 18, when the patient expected it. The patient died August 24.

Case 92.—A woman, aged 49 years, weighing 215 pounds, had bleeding uterine fibroids, marked secondary anemia, and a degenerating colloid goiter. She was

given three transfusions, two of which were of Group IV blood, after which a partial thyroidectomy was done. Menstruation began 11 days after the last Group IV transfusion, but stopped immediately, although previously bleeding had been profuse.



TEXT-FIG. 5. Curves illustrating the drop in transfused blood that takes place at the menstrual period. ↕ indicates the beginning of a menstrual period; ↕ indicates the time at which menstruation should have occurred. ——— Unagglutinated corpuscles. – – – Total red blood count. ↕ indicates a Group IV transfusion; ↓ a like group transfusion.

Case 103.—A woman, aged 32 years, had pernicious anemia and received a series of transfusions. While under observation profuse menstruation took place, beginning the 24th day after the Group IV transfusion.

In examining these curves it must be borne in mind that several factors other than blood elimination will bring about changes in the corpuscle count. Of these the most important is due to dilution from increase in blood volume. Robertson and Bock have shown, and my experience corroborates theirs, that after hemorrhage there is often only a slow return to normal blood volume. It is probable, too, that changes in the capillary circulation cause changes in the blood count taken from the ear which do not represent changes in the average corpuscle content. Robertson and Bock have shown that in cases in which the blood volume was low the difference between the capillary and vein hemoglobin might be as much as 12 per cent of the vein hemoglobin. There seem also to be daily changes in the blood volume in certain patients. Curves are very much smoother in which the counts have all been made at the same time of day.

It will be seen in Text-fig. 2 that no definite elimination is shown in the curves there represented. In Case 95 there was no change that could be interpreted as an elimination of transfused blood in 51 days. In Case 115 after a slight initial drop, presumably from blood volume adjustment, the count was constant within errors of technique for 33 days. In this instance the counts were taken at the same time of day. In Case 93 the patient was under observation for 43 days, with no definite change other than the initial blood volume adjustment and adjustment after severe vomiting. In Case 97 there was no elimination in 51 days. As these curves maintain a level for a long time, indicating that during that time there was no elimination, it is obvious that, as the blood is eventually eliminated, it is not eliminated by a continuous process but by an intermittent process.

Text-fig. 3 shows two curves of elimination of transfused blood of men, one normal and the other with purpura of several years standing, but in fairly good health, and the third, of a woman beyond the menopause, who was jaundiced. It will be seen that in these curves there are periods of elimination which are short and probably would have proved to be shorter if the counts had been taken closer

together. The first drop in Case 119 took place between two counts spaced 2 days apart. The drop in Case 55 took place between counts that were taken 3 days apart. The first drop in Case 116 took place between two counts that were 5 days apart; the apparent further drop on the following day is due to a change in technicians. It would seem, then, that these drops take place rapidly.

It will be seen that there is a periodicity, although a somewhat irregular one, in the period of elimination of the two men, ranging in the normal man from 18 to 28 or 30 days. Accompanying these drops there tends to be an increase in the total blood count.

On examining the nature of the falls in the curves it will be seen that they are such as to suggest a blood-destroying activity on the part of the body rather than a spontaneous disintegration of simultaneously formed corpuscles. For if we postulate a blood destruction which is dependent solely on the condition of degeneracy of the corpuscle, we shall have to assume not only that blood formation takes place in short periods, which may be the case, although the continued presence of reticulated corpuscles would seem to indicate that there is at least some continuous formation, but also that the individual corpuscles are so uniform that the wear and tear which they experience after 3 months (Case 119) in the circulation bring them to a simultaneous disintegration point. But even if we are willing to make this assumption, further examination of the figures does not bear out this hypothesis. For if the condition of wear and tear were wholly responsible for initiating the destruction of the corpuscles, we should expect a regularity in the rate of their disappearance. If, for instance, the corpuscles which were transfused had been formed in sharp crises every 3 weeks and their life were 12 weeks, then one-fourth of the corpuscles was due to live 12 weeks, one-fourth 9 weeks, one-fourth 6 weeks, and so on. Equal amounts should have disappeared at each period of elimination. But this was not the case; by far the largest fall came in the first periods of elimination. In Case 119 the first drop was 200,000, or nearly one-half of the blood transfused, while subsequent drops were each approximately 80,000, or one-fifth of the original blood injected. In Case 116 the first drop was 150,000, or three-eighths of the blood injected, while subsequent drops were 90,000 and 80,000, about two-tenths

of the injected blood. These figures seem to place the elimination with reference to some active process on the part of the body, probably modified by the condition of the corpuscles and the number present in the circulation.

In Text-fig. 4 are included the curves of the three women studied who received Group IV transfusions immediately before or subsequent to hysterectomy. In each case a period of elimination came between the 25th and 30th days after hysterectomy. The elimination would probably have proved to be sharper if the counts had been taken closer together. In one of these women a second period of elimination occurred about 20 days after the first. This suggests, although the series is too short to have a conclusion drawn from it, that in some way the hysterectomy initiated a cycle equivalent to the menstrual cycle, and when complete elimination did not take place the subsequent periodicity of elimination was irregular as in the case of the two men.

Text-fig. 5 summarizes all the data that I have been able to obtain on the relationship between the elimination of transfused blood and the menstrual period. Six menstruations, and two periods when menstruation should have occurred but did not, were observed. There has been no exception to the fact that elimination of transfused blood that could not be accounted for by simple blood loss from the menstrual flow occurred during the menstrual period. It has not always happened that when menstruation should have occurred but did not that there was an elimination of transfused blood. In the two cases in which the elimination did occur without the appearance of the menstrual flow, the women were in a condition of extreme anemia, and it is probable that the suppression of the flow was due to the anemia rather than to the suspension of the menstrual process.

In Case 115, which was the most intensive study made, the patient's blood volume was low and her count showed a great variation during the day, being much higher in the morning than in the evening, so that only counts taken at the same time of day were comparable. Those taken through the menstrual period that came 32 days after transfusion were made in the morning by one observer; those during the menstrual period that came 58 days after transfusion were made

by a second observer, and in the evening. The count which determines the further end of the line connecting the two menstrual periods was made by the first observer at 1 p.m. The nearer end of the line is determined from a calculated figure. It will be noted that during the earlier menstrual period, although there was a decrease in the total red blood cell count, there was some increase in the hemoglobin. At this time the transfused blood had a higher index than the patient's blood, being 0.7 per cent, while the patient's blood was 0.5. During the later menstrual period there was a rise in the total blood count. In Case 38, with a very slight menstruation, there was an elimination of more than half the transfused blood; the preliminary fall in this curve is due to blood volume adjustment. There had been massive hemorrhage, and the patient's blood volume was low. In Case 103, that of a pernicious anemia patient, although the menstrual flow was profuse, there was a moderate fall in the transfused blood and a slight increase in the total count. In Case 48, in which menstruation did not occur when it was due, there was a complete elimination of the transfused blood with a slight temporary rise in the total blood count. In Case 68, one of pernicious anemia, a big fall in the Group IV transfused blood occurred beginning the 21st day, which may have been in part due to a blood volume improvement, since the patient's blood volume had been exceedingly low, and her general condition in the face of what seemed to be a period of blood loss became no worse. There was, however, at this time a marked elimination of the native corpuscles and of like group corpuscles, since the difference between the patient's whole blood count and the count of the transfused blood became so slight that it was within the limit of experimental error. From the 21st to the 25th days the difference between the two counts increased again, which in view of the practically complete elimination of the patient's own blood must indicate a coincident blood production.

DISCUSSION.

There seems to be little doubt that the elimination of Group IV transfused blood is brought about by an active destroying process of the body which is some part of a metabolic cycle, evidenced in

women by menstruation. Whether the Group IV transfused blood is eliminated as part of a general blood-eliminating effort of the body or whether it is eliminated as a foreign body is a matter of interest. The fact that the Group IV transfused blood stays in the circulation for considerable lengths of time would be an argument against its foreign relationship to the body, but, on the other hand, in my studies of Group IV transfusions in patients with pernicious anemia, the Group IV transfused blood has remained in the circulation in the presence of destruction of the patient's own blood, and in one instance, Case 68, in the presence of destruction of transfused blood of the same group as the patient's. It would seem that if the elimination of the Group IV blood takes place because of a normal blood-eliminating process, either there must be two processes, one of which can handle the blood of the unlike group, while the other cannot, or else that a certain pressure of elimination must be attained before the threshold is reached for the elimination of Group IV which is apparently more difficult to destroy. Unless it is assumed that some Group IV transfusions are, relative to the patient's own blood, less enduring than others, the latter would not seem to be the case because in some instances we have seen complete elimination of the transfused blood in one elimination period, which would, on the assumption of a higher threshold mechanism, necessitate tremendous coincident elimination of the recipient's blood. If we assume two processes at work in the physiologic elimination of blood, one of which is more able to handle the apparently slightly foreign Group IV blood, we might conceive of it as part of a more general lytic or phagocytic function which has as one of its expressions of activity the sloughing of the endometrium and which is able to overstep the group differences. On the other hand, this periodic elimination of transfused blood may not be due to any specific blood-eliminative activity, but to a non-specific eliminative activity.

It would be a matter of great interest to know whether or not normal physiologic blood elimination also takes place in sharp cyclic crises. Satisfactory evidence on this point is difficult to obtain because if there is such blood elimination it may be masked by production, or if there is any apparent drop in the total blood count we do not at present know that it is not due to changes in blood volume or to an uneven distribution of corpuscles.

Carnot and Deflandre, Pölzl, and Sfameni, who studied the changes in the red corpuscle count over the menstrual cycle, report an increase in the count before the menstrual period, with a fall upon the onset of menstruation, which fall has no relationship to the amount of blood lost in the menstrual flow. In anemic women an improvement in the blood was noted after menstruation. Carnot and Deflandre point out that the fall may be only apparent, due to the accumulation of corpuscles in the congested pelvic organs, but call attention to the various toxic phenomena which accompany menstruation and the vitality which follows the period. It is generally conceded that menstruation is not a local but a generalized process. The numerous instances of vicarious menstruation seem to place this point beyond discussion, and there is in addition supporting evidence derived from metabolic studies, such as creatine excretion, nitrogen retention, reported by Schrader, blood calcium reported by Bell, changes in blood cholesterol reported by Gonalons, and increase in basal metabolic rate reported by Ford. The fact that some generalized change takes place at this time and with it a destruction of transfused blood is strongly suggestive that the drop in the red count reported is also due to a corpuscle destruction process rather than to a change in blood volume or an uneven distribution of corpuscles. The fact, too, that the blood picture of anemic women has been observed definitely to improve after a menstruation, in addition to being evidence in favor of the crisis in corpuscle production, supports the idea of a coincident crisis of destruction.

SUMMARY AND CONCLUSIONS.

Group IV transfused blood in a recipient of unlike group is eliminated by a blood-destroying activity of the body.

This blood-destroying activity is periodic both in men and women, and in women coincident with menstruation.

The elimination of the transfused blood probably takes place as part of a period of blood-destroying and blood-producing activity of the body, although direct evidence to this effect is so far lacking.

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II. Blood Destruction in Pernicious Anemia.

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STUDY OF TRANSFUSED BLOOD.

II. BLOOD DESTRUCTION IN PERNICIOUS ANEMIA.

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Many of the older writers inclined to the idea that pernicious anemia is fundamentally a failure in erythrogenesis, but in the light of recent investigation it is more generally assumed that increased blood destruction is the important cause of the anemia seen in this disease. This assumption rests mainly upon increase in hemosiderin deposits in the tissues, increase in bile pigment output, the rapid falls that are seen in the blood count, and the anemia in the face of what is apparently a hyperplasia of the bone marrow. My own results of the study of thirty-three cases of pernicious anemia patients who received Group IV transfusions have not realized the intensive blood destruction that I expected to find.

Upon going over the literature of the subject I find it possible to put more than one interpretation upon these facts of increased bile pigment excretion, hemosiderin deposits, etc., which are usually assumed to be evidence of a high rate of blood destruction in pernicious anemia. The increased activity of the bone marrow as judged by the increased density of mitotic figures and immature cells and the increased mass of erythropoietic tissue may be only apparent. If there were some factor present which retarded the speed of the process of maturation and division, while the physiologic stimulus to erythrocyte production brought into play by the anemia caused an increase in the number of cells dividing, we might have such a picture with a decrease in the total cell output. The hemosiderin deposit is evidence only that iron is not being kept in circulation. This may be due to abnormal destruction of hemoglobin, but it is equally possible that it is due to a failure to utilize sufficiently quickly

in the formation of new hemoglobin, iron released by a normal rate of blood destruction. Increased bile pigment output cannot be accepted as evidence of increased blood destruction because Whipple and Hooper have shown that bile pigment is derived from other sources besides hemoglobin. It is conceivable that with a depression in protein synthesis the pigment material which might have combined with globin to form hemoglobin is diverted to the increase of the bile pigment output. Urobilin and urobilinogen in the duodenal content and urine, although they may appear in these excretions when there is blood destruction, are only evidence that bilirubin has been reduced before it reaches the intestinal tract or that it was incompletely synthesized. Rapid falls in the blood count can just as plausibly be explained on the basis of a failure of production and the physiologic end of the corpuscle as of its abnormal destruction. All these facts of hyperplastic bone marrow, increased bile pigment, etc., which from one angle seem to point collectively towards excessive blood destruction can, by changing the point of view, be made to present just as solid a front against it. I do not feel that our laboratory studies of pernicious anemia offer anything that can be accepted as absolute proof of excessive blood destruction.

Perhaps we have our best evidence of blood destruction in pernicious anemia when upon transfusion the total count fails to increase or even decreases and when the symptoms of the patient do not indicate any blood volume improvement. Since it is now believed that there is no intrinsic destructibility of transfused blood, even of citrated transfused blood, this failure of the corpuscle content to increase when corpuscles are injected is evidence of corpuscle destruction. This situation, however, is the occasional one and, unless the patient succumbs, is only temporary. It is evidence that there are at least intermittent periods of destruction, but it does not support the idea which some investigators have of a continuous destruction.

On the assumption of an increased blood destruction, the prevailing opinions as to its cause cover all the possibilities. Most of these can be included under three headings. We can conceive of an extraneous hemolytic toxin as causing injury to the blood corpuscles, resulting in their destruction, either by disintegration in the circulation or

by later elimination by the body. It is conceivable that a toxin is produced which does not act directly upon the blood cell in the circulation, but affects the bone marrow so that a type of corpuscle is produced which either becomes effete more rapidly and, in consequence disintegrates or is removed from the circulation, or which deviates from normal blood to such an extent that it is removed because of its foreign quality. Lastly, it is conceivable that without any injury to the corpuscles in the circulation or to corpuscle production, there might be a speeding up of the physiologic blood destruction agencies. It is also conceivable that all these effects might be produced not by a specific toxin, but by the toxic effect of some abnormal metabolic habit brought about by some past injury.

The most popular conception of the cause of blood destruction in pernicious anemia is the hemolytic toxin. Wells, in the new edition of his Chemical pathology, says: "Putting together the above findings, we see that in pernicious anemia we have every evidence that excessive hemolysis is taking place, and the fact that continued poisoning by toluylendiamine and other hemolytic poisons, such as that of *Bothriocephalus*, may give rise to a condition resembling pernicious anemia very closely, indicates strongly that hemolytic poisons are the cause of pernicious anemia."

Pappenheim says that other "hyperchrome" anemias, like pernicious anemias and hemolytic anemias, are at the same time hemotoxic, myelotoxic, and splenopathic, but for all these manifestations of the disease a single toxin, as in the case of toluylenediamine, may serve. He is convinced that in all cases the "hyperchrome" anemia is due to a toxin. He points out that these poisons, such as toluylenediamine and pyrodine, which produce the "hyperchrome" anemia, are not hemolytic. Pyrodine, for instance, does not always produce the "hyperchrome" anemia, but sometimes first kills the animal from general organ poisoning. When the "hyperchrome" anemia is produced, Pappenheim considers that it is due to a tendency of the animal organism to transform the pyrodine into hydroxylamine, which is a hemolytic substance. He thinks it probable that the various conditions, lues, purpura, pregnancy, *Bothriocephalus* infection, etc., which seem sometimes to cause pernicious anemia, do so because they establish some improper metabolic process, perhaps by injury to some unknown organ of internal secretion, so that the body forms the hemolytic toxin, hydroxylamine.

Bunting, who produced a condition resembling pernicious anemia with continued small doses of ricin, considers it probable that the disease is due to the long continued action of a hemolytic poison, which produces the blood picture by causing an overstimulation of the bone marrow. Hunter, Herter, and others found hemolytic substances in the intestinal tract and considered the anemia due to their absorption, but no experimental work establishes their view.

The production by Schauman and Tallquist of *Bothriocephalus* anemia in dogs with hemolytic extracts from the worm rests on too meager evidence to be accepted without further work. These investigators treated seven dogs with worm extract and pieces of worms and produced only slight changes in the blood in all but one, which died with a blood count of 3,400,000 and without typical changes in the blood picture. The claim is made that these dogs clinically showed symptoms of anemia, but no clinical symptoms typical of pernicious anemia are reported. Blanchard quotes Vlaiev's negative attempts to produce anemia in rabbits and pigeons with extracts of *Bothriocephalus* tapeworm. Seyderhelm, working with the bot-fly in horses, reports that he was able to reproduce the typical anemia, not with the extract, which proved to be hemolytic, but with an alcohol-insoluble material which *in vitro* has no solvent effect on red blood corpuscles.

Except for Bunting's ricin, which besides being hemolytic has other strongly toxic properties, the establishment of anything resembling pernicious anemia with a hemolytic toxin does not seem to have been clean-cut.

Among the men who incline to the idea that the blood destruction in pernicious anemia is due to incompatibility or low resistance of the corpuscles brought about by a toxin are Gulland and Goodall, and Hirschfeld. MacCarty, of this Clinic, considers it probable that the erythrogenic process has become malignant and that the corpuscles are eliminated rapidly from the circulation because of their foreign quality. We have no experimental proof of a lowered resistance of the corpuscle of pernicious anemia.

The possibility of an excessive activity of the physiologic blood-destructive process of the body is not emphasized in the literature, although mention is made of it. The idea that sepsis is the cause of pernicious anemia may be grounded in such a conception. Sajous points out that hemolysis occurs whenever toxins, either endogenous or exogenous, appear in the blood in quantities sufficient to excite a defensive reaction. The antibodies then attack not only the toxins, but the erythrocytes as well. Clark and Evans suggest the interesting possibility that the anemia of the so called hemolytic anemias may be due to a reduction in the power of the blood serum to protect the corpuscles from whatever hemolytic agencies they may come in contact with normally in the circulation and present experimental evidence that in these anemias there is a reduction of the protective power of the serum against sodium oleate.

The following attempt was made to obtain some understanding of the mechanism by which blood destruction is brought about, by means of the study of the elimination of Group IV transfused blood. The technique used was that already described. Thirty-three patients with pernicious anemia not of Group IV were given Group IV transfusions. Four of them were under observation for about 3 months, ten were observed for 1 month or more, eight for at least 17 days, and the rest for shorter periods of time.

The object of the study was first to answer the question as to whether or not there is any hemolytic toxin at work in pernicious anemia by comparing the curves of elimination of Group IV transfused blood in these cases with those obtained from patients without any blood disease. As a control, patients with other diseases, in which it was suspected that there might be increased blood destruction, were chosen for transfusion.

It was hoped that if no excessive destruction of Group IV blood resulted the study might produce data that would give some clue as to which of the other two possibilities, namely the intrinsic weakness of the corpuscle or the speeding up of normal blood destruction, is responsible for the anemia.

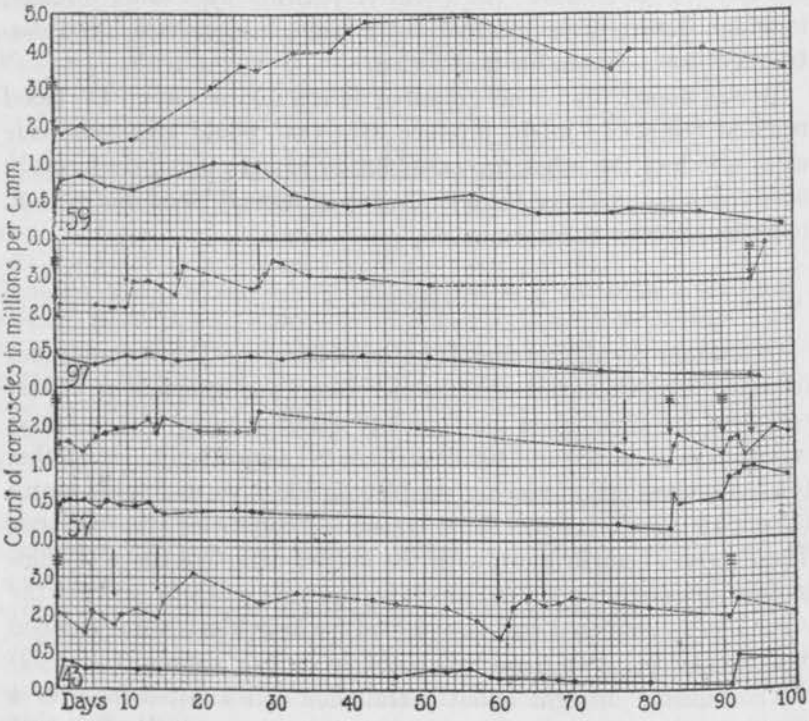
RESULTS.

Of the four pernicious anemia patients (Text-fig. 1) who were studied until the elimination of transfused blood was complete or almost complete, the time taken for complete elimination in two instances was 91 days (Case 43) and 100 days (Case 59) respectively. In another (Case 97) elimination was not complete in 100 days, and in the fourth (Case 57) there were still some transfused corpuscles after 83 days. These curves compare well with the longest complete curve of elimination obtained in a normal individual and are much longer than the other seven completed curves obtained for individuals without blood diseases (Ashby). Of these four patients, in one instance (Case 59) the transfusion brought about a remission which lasted for over a year. In the other three cases it was necessary to give repeated transfusions during the time that the patients were under observation.

Case 43.—The patient was a man, aged 49 years, weighing 163 pounds. He had no free hydrochloric acid in the stomach. He had glossitis and combined sclerosis of the cord. The red cell count was 1,900,000, with a hemoglobin of 44 per cent, and color index of 1.4+. The white count was 3,400, with 36 per cent lymphocytes. There was marked anisocytosis and moderate poikilocytosis. Diagnosis of pernicious anemia was made. A series of six 500 cc. transfusions of citrated blood was given.

Case 57.—The patient was a man, aged 63 years, weighing 157 pounds. He complained of a general increase in weakness, beginning 2 years ago, when he became sallow, lost his appetite, and his legs swelled. There were glossitis, and

numbness and tingling in the feet. The red blood count was 1,136,000, hemoglobin 25 per cent, color index 1.1+, leucocytes 4,200, polynuclear neutrophils 44 per cent, lymphocytes 56.5 per cent, normoblasts 3 per cent; marked anisocytosis and poikilocytosis. Diagnosis of pernicious anemia was made. A series of eight 500 cc. transfusions of citrated blood was given.



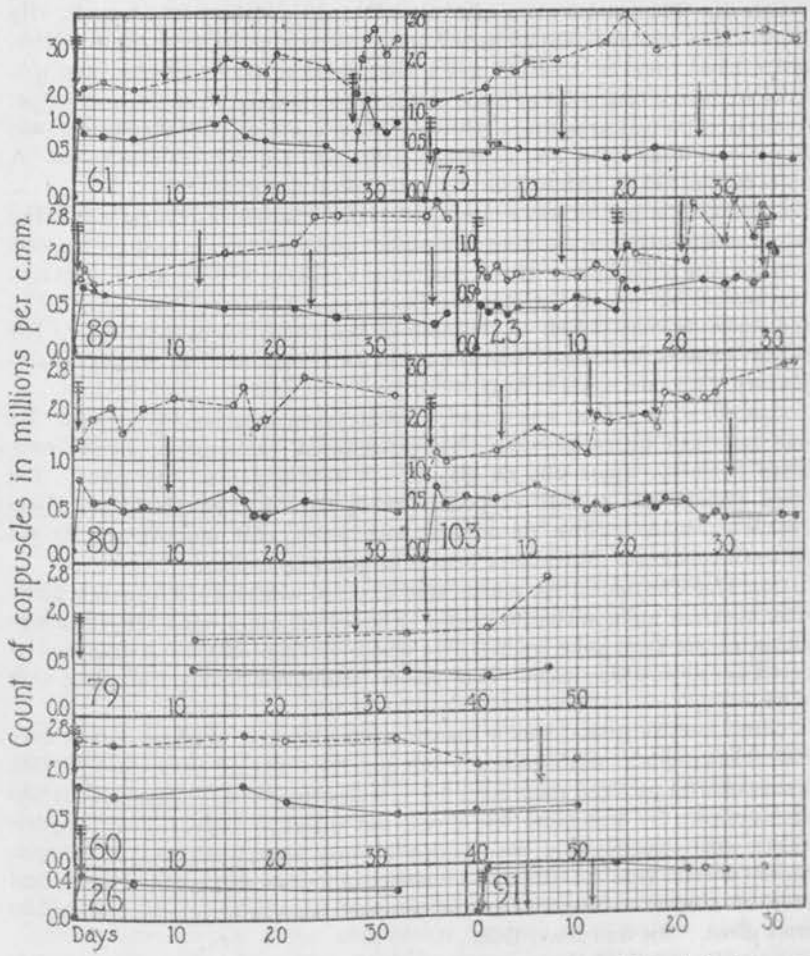
TEXT-FIG. 1. The curves of elimination of 500 cc. of Group IV transfused blood in four pernicious anemia patients, studied until elimination was complete or nearly complete.

——— Unagglutinated corpuscles. - - - - Total red blood count. ↕ indicates a Group IV transfusion; ↓ a like group transfusion.

Case 59.—The patient was a short woman, aged 52 years, weighing 149 pounds. In February, 1918, when she first came up for examination, she was sallow and weak, had numbness of hands and feet, and a smooth tongue. Her red blood count was 1,900,000, color index 1.1+, leucocytes 3,400, and lymphocytes 72 per cent. There were moderate anisocytosis and poikilocytosis. Diagnosis of pernicious anemia was made. She was given 2,500 cc. of blood in three transfusions in the course of a month; this was followed 2 months later by two 500 cc. trans-

fusions. In May, 1919, she returned, with a red blood count of 1,330,000 and was given one 500 cc. Group IV transfusion, after which she improved and maintained a fairly good count to date.

Case 97.—The patient was a man, aged 42 years, weighing 155 pounds. His chief complaint was weakness. He had been gradually losing strength for 3



TEXT-FIG. 2. Elimination curves of Group IV transfused blood in ten pernicious anemia patients under observation for 30 days or more. ——— Unagglutinated corpuscles. - - - Total red blood count. ↧ indicates a Group IV transfusion; ↓ a like group transfusion.

years. He had glossitis, absence of free hydrochloric acid in the stomach, and tingling and numbness of the arms and legs. His red blood count was 2,240,000, hemoglobin 50 per cent, color index 1.1−, and leucocyte count 4,000. The diagnosis made was pernicious anemia. The patient received a series of five (500 cc.) transfusions, of citrated blood. Later he returned for further transfusions.

Case 23.—The patient was a man, aged 57 years, weighing 139½ pounds. He suffered from weakness, dating back 7 years. 2 years previous to present date, with a blood count of 3,500,000 and a color index of 0.9+, glossitis, and posterior sclerosis, a diagnosis was made of pernicious anemia, of the neurotic type. A year later the patient returned, with a red blood count of 1,090,000, hemoglobin 26 per cent, color index 1+, leucocytes 3,800, and red cell count 830,000. A series of four 500 cc. transfusions of citrated blood was given.

Case 26.—The patient was a man, aged 44 years, weighing 148½ pounds. The skin was lemon-yellow, the tongue atrophic, and there was a systolic murmur over the base of the heart. He complained of weakness, which began 7 years ago. The hemoglobin was 32 per cent, red blood count 1,480,000, color index 1.1+, and leucocyte count 2,600. A diagnosis of pernicious anemia was made. The patient received a series of transfusions in 1918. He received transfusions in January, 1919, and again in May, 1919.

Case 60.—The patient was a man, aged 36 years, weighing 137 pounds. He was pale, and extremely weak. The neurological examination showed combined sclerosis. There was no glossitis. A test meal was not given. The blood picture was typical of pernicious anemia. A diagnosis of pernicious anemia was made. Three transfusions were given, with improvement in the patient's condition. There have been subsequent remissions.

Case 61.—The patient was a man, aged 42 years, weighing 152 pounds. There were weakness and combined sclerosis. The red cell count was 1,510,000, hemoglobin 34 per cent, color index 1.1+, leucocyte count 2,000. A diagnosis of pernicious anemia was made. Four 500 cc. transfusions of citrated blood were given.

Case 73.—The patient was a woman, aged 47 years, weighing 110 pounds. Her chief complaint was weakness. July 28, 1919. The red count was 1,750,000, hemoglobin 34 per cent, color index 0.9−, leucocytes 5,000. There was marked anisocytosis. No test meal was given, but digestion improved with hydrochloric acid. Neurological examination showed spinal sclerosis of the pernicious anemia type. A diagnosis of pernicious anemia was made. The patient received a series of transfusions and returned home, where more transfusions were given. She died May, 1920.

Case 79.—The patient was a man, aged 25 years, weighing 126 pounds. He had a palpable spleen, and there was no free hydrochloric acid in the stomach. The neurological examination showed nothing, and there was no evidence of glossitis. His red cell count was 1,120,000, hemoglobin 23 per cent, color index 1,

leucocyte count 4,800, neutrophilic myelocytes 4.5 per cent, normoblast 4 in a count of 200, and megaloblasts 1. Diagnosis of pernicious anemia was made. Three 500 cc. transfusions of citrated blood were given. Since then the patient has returned twice for transfusions.

Case 80.—The patient was a man, aged 34 years, weighing 154 pounds. He had a bald tongue, but no evidence of combined sclerosis. His chief complaint was weakness and indigestion. The red cell count was 1,960,000, hemoglobin 40 per cent, color index 1+, leucocyte count 6,400, with small lymphocytes 48 per cent. There were moderate anisocytosis and poikilocytosis. Diagnosis of pernicious anemia was made. (On the 15th day after transfusion the patient took magnesium sulfate for stool examination.) Two transfusions of 500 cc. of citrated blood were given.

Case 89.—The patient was a woman, aged 60 years, who normally weighed 180 pounds and at time of examination weighed 127 pounds. Her chief complaint was weakness. Transfusions elsewhere had improved her condition. She had pigmented skin, sore tongue, and no free acid in the stomach. The red blood count was 1,820,000, hemoglobin 38 per cent, and color index 1. The leucocytes were 6,200, lymphocytes 33.5 per cent, and normoblasts 1 per cent. There were marked anisocytosis and poikilocytosis. Diagnosis of pernicious anemia was made. A series of four transfusions of citrated blood (500 cc.) was given.

Case 91.—The patient was a woman, aged 53 years, weighing 137 pounds. She showed marked weakness, glossitis, dyspnea, short pulmonic systolic murmur, edema, and early symptoms of combined sclerosis. Her color was lemon-yellow. Red blood cell count was 1,440,000, hemoglobin 35 per cent, color index 1.24, leucocytes 6,600, with polynuclear neutrophils 56 per cent, small lymphocytes 39.5 per cent, large lymphocytes 2.5 per cent, eosinophils 1.5 per cent, neutrophilic myelocytes 0.5 per cent, normoblasts 1.5 per cent, megaloblasts 0.5 per cent; moderate anisocytosis and poikilocytosis. The diagnosis was pernicious anemia. Three transfusions (500 cc.) of citrated blood were given.

Case 103.—The patient was a woman, aged 52 years. She complained of weakness. She was well until a year before, when she had influenza. There were glossitis, posterolateral sclerosis, soft systolic murmur at the apex of the heart transmitted to the axilla, and retinitis of pernicious anemia. The spleen was palpable 1 inch. The red cell count was 960,000, hemoglobin 23 per cent, color index 1.2+, leucocytes 2,400, polynuclear neutrophils 13.5 per cent, small lymphocytes 78 per cent, large lymphocytes 8 per cent, neutrophilic myelocytes 0.5 per cent, normoblasts 12 per cent, megaloblasts 1 per cent; marked anisocytosis and poikilocytosis. While under observation profuse menstruation took place, beginning December 20, 1919. A series of four transfusions of citrated blood (500 cc.) was given.

In the curves in Text-fig. 2 it will be seen that in no case is there any elimination of Group IV transfused blood that cannot be du-

plicated in the control cases with no blood disease (Ashby), and the majority do not show any drop as great as that which occurred in normal individuals. In Case 89 the fall up to the 22nd day would seem to be blood volume improvement, as the patient's blood volume, calculated from the transfusion data immediately after transfusion, was low, being 6.4 per cent of the body weight, whereas the blood volume in secondary anemia as found by this method is 8 to 9 per cent of the body weight. The same may be said of the apparent fall in Case 60. In Case 26 the count of unagglutinated corpuscles with a blood volume of 8.5 per cent would have been 342,000, so the count of this patient on the 6th day after transfusion was still more than the number that would have been present if a normal blood volume adjustment had been attained. As there was no count taken between the 6th and 32nd days, the patient having gone home in the meantime, we have no data as to when the drop which occurred in the curve between those dates took place. We have no reason, however, to assume that it was any sooner than would have been normal.

Group IV blood transfusions in seven cases of pernicious anemia were studied over periods varying from 15 to 26 days. These protocols have not been included to save space. In a case studied for 28 days two Group IV transfusions were given during that time. At the end of the period there were 934,000 Group IV corpuscles per c.mm. in the circulation. The initial Group IV transfusion had given 488,000 Group IV corpuscles per c.mm. The count of 934,000 would seem to account for two transfusions. In a case studied for 24 days the count of unagglutinable corpuscles fluctuated between 738,000 and 596,000 for the first 3 days after transfusion. After 24 days, the count was 627,000. A case studied for 20 days showed an initial count of 509,000 Group IV corpuscles and a final count of 507,000. Two cases studied for 19 days showed in one instance no elimination, as the initial Group IV corpuscle count was 436,000 and the final, 428,000. In the other instance there may have been some elimination between the 17th and 18th days, as the count changed from 440,000 to 389,000, although judging from previous fluctuations and the fact that the condition of the patient markedly improved although the total red blood count also dropped

somewhat it would seem possible that this drop was due to a blood volume increase. A case studied for 15 days gave an unagglutinated count of 548,000 on the 2nd day after transfusion and 542,000 on the 15th day. In none of these cases does there appear to have been any elimination of Group IV transfused blood during the periods studied, except perhaps in the case that was studied for 18 days, and here the decrease in count, if it was due to elimination and not to blood volume change, can be duplicated in a normal curve. Eight other cases studied for shorter lengths of time showed no elimination of the Group IV transfused blood.

In contrast to the stability of transfused blood in pernicious anemia cases is the elimination which was found to take place in certain patients with other conditions. In the cases which came under observation during the course of this study five appear to have shown a progressive and rapid elimination of Group IV transfused blood. This feature was most markedly shown in Case 118, one of hemolytic jaundice, in which there was a continuous fall in the curve of transfused blood until splenectomy, when the fall ceased sharply. Two cases of aplastic anemia (Cases 81 and 102, Text-fig. 3) one of myelogenous leucemia (Case 39), and one of carcinoma of the kidney with metastasis show rapid elimination of the transfused blood. The protocols and chart follow.

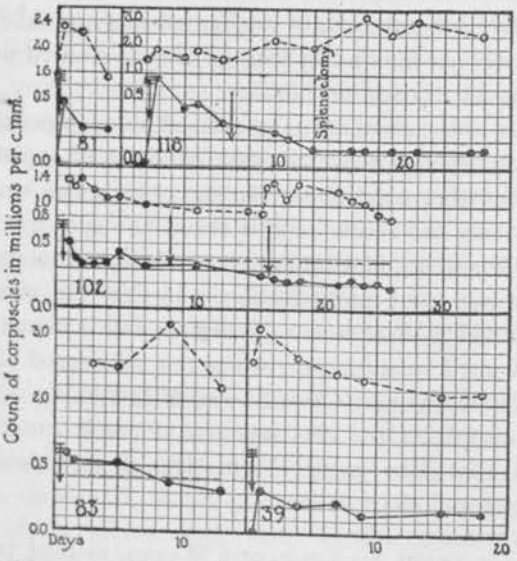
Case 39.—The patient was a man, aged 55 years, weighing 166 pounds. He had myelogenous leucemia with a white blood count of 215,000. A transfusion was given, followed after 6 days by a radium treatment. Profuse sweating occurred the 15th day after transfusion, with edema of the eyes and forehead; on the 18th day the edema was extreme. The patient died on the 19th day.

Case 81.—A man, aged 45 years, weighing 154 pounds. Diagnosis of aplastic anemia was made. The patient died August 29, 1919, of bilateral bronchopneumonia on the 4th day after transfusion. There were miliary tubercles in the spleen.

Case 83.—The patient was a man, aged 69 years, weighing 105 pounds. There was carcinoma of the left kidney, with metastasis. A left nephrectomy was followed by a transfusion with 500 cc. of Group IV citrated blood.

Case 102.—The patient was a man, aged 28 years, weighing 124 pounds. In October, 1918, he had influenza, which was followed by weakness and bleeding. There was severe epistaxis in October, 1919. Upon examination in November, the red blood count was 1,160,000, and the hemoglobin 13 per cent, with a color index of 0.5+. The leucocytes were 10,800, with polynuclear neutrophils 25

per cent, small lymphocytes 37.5 per cent, large lymphocytes 35.5 per cent, eosinophils 0.5 per cent, basophils 0.5 per cent, and neutrophilic myelocytes 1 per cent. There was moderate anisocytosis, and the platelet count was 134,000. December 9. The platelet count was 96,000. A diagnosis of aplastic anemia was made. Four transfusions were given. December 12. An eruption appeared upon the arms and face, which proved to be smallpox. January 1, 1920. The patient died.



TEXT-FIG. 3. Control cases in which there appears to have been some progressive elimination of Group IV transfused blood. ——— Unagglutinated corpuscles. – – – Total red blood count. – – – – – The count of unagglutinable corpuscles that would be expected in an individual the size of a patient with a blood volume of 8.8 per cent of the body weight. ↧ indicates a Group IV transfusion; ↓ a like group transfusion.

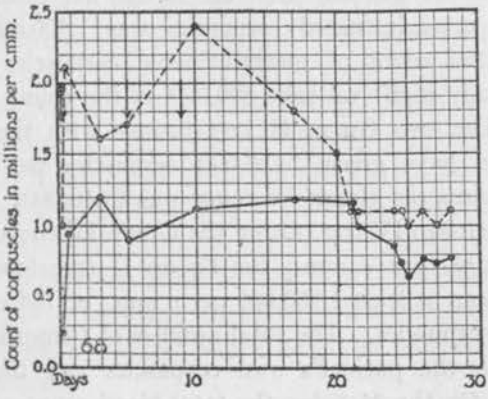
Case 116.—The patient was a boy, aged 13 years, weighing 76½ pounds. He was jaundiced and weak. He had an enlarged spleen, which had been first noticed 3 months previously. The red blood count was 1,750,000, and hemoglobin 30 per cent, giving a color index of 0.8+. The white cell count was 19,500, with 65 per cent polynuclears and 25 per cent lymphocytes. The platelets were 148,000, and the bleeding time ½ minute. There was increased fragility. The blood picture did not improve after two transfusions. There was no evidence of familial jaundice. A diagnosis of hemolytic jaundice was made. Splenectomy was performed and the patient regained normal health.

When the curves of the total red blood counts of the pernicious anemia patients are examined, it will be seen that, although in most cases it would be difficult to say that there has been blood destruction during the preceding transfusion studies, there are some instances in which blood destruction is definite. In estimating these curves it must be borne in mind that changes in the blood volume very materially influence the total count. Changes in the number of transfused blood corpuscles per cubic millimeter give some indication of changes in blood volume. When the transfused blood count rises it is probable that the blood volume decreases, and when the transfused blood count falls with a subsequent return, showing that the fall was not due to elimination, it is probable that the blood volume increases. This has been assumed in the following calculations.

In Case 23 on the 10th day after the initial Group IV transfusion the total count was 1,100,000, the Group IV transfused blood was 520,000 minus the initial unagglutination of 70,000, or 450,000. Since the subsequent Group II transfusion 2 days previously was 100 cc. larger than the Group IV transfusion, it should account for at least the same number of corpuscles. The total count before this transfusion had been 1,170,000 with apparently a larger blood volume, as the count of the unagglutinable corpuscles was less. There have been added at least 450,000 corpuscles per c.mm., the blood volume has decreased, which should have still further increased the total red cell count, but the total count is still what it was before transfusion. We have here evidence of destruction. In Case 61 on the 15th day after the Group IV transfusion the total count was 2,250,000. The Group IV corpuscles were 700,000, so the two like group transfusions which had been given on the 9th and 14th days should have added 1,400,000, or twice that amount. The total count 3 days before these transfusions was 1,570,000, when the blood volume, judging from the unagglutinable corpuscle count, was somewhat higher; 1,400,000 corpuscles per c.mm. have been added to the blood, the blood volume has decreased, which would still further increase the count, and the total count has increased only 680,000. Here there is a loss of over 700,000 corpuscles per c.mm. within 8 days. In Case 73 on the 20th day after the Group IV

count was 1,060,000, hemoglobin 28 per cent, color index 1.3, leucocyte count 7,400, lymphocytes 74.5 per cent. In counting 200 white cells, 89 normoblasts and 3 megaloblasts were seen. There were marked anisocytosis and poikilocytosis. Menstruation was expected on the 21st day after the transfusion but did not occur. The patient died a month later.

We have two cases (Nos. 64 and 49) which appear to be exceptions to the rule that in the pernicious anemia patients so far studied there is no increased rate of elimination of Group IV transfused blood. In Case 64 when the total red blood count was 1,400,000 a 500 cc. Group IV transfusion was given. The



TEXT-FIG. 4. Curves of a patient who showed elimination of the Group II transfused corpuscles as well as elimination of her own corpuscles. ——— Unagglutinated corpuscles. - - - - - Total red blood count. ↧ indicates a Group IV transfusion; ↓ a like group transfusion.

next day the count of unagglutinable corpuscles was 600,000. 5 days later when a second count was made the unagglutinable corpuscles had dropped to 99,000, which meant practically complete elimination of the transfused blood. The patient's total count was then 1,100,000. A Group II transfusion was given and 4 days later the total red blood count was 700,000. The patient died within a month. In Case 49 with the patient's initial blood count at 1,100,000 a 500 cc. Group IV transfusion was given, which gave 620,000 unagglutinated corpuscles per c. mm. and raised the total count to 1,500,000. The next day the unagglutinated count was 170,000

while the total count was 1,200,000. On the following day the unagglutinated count fell to 50,000, which was practically complete elimination, with a return in the total count to 1,100,000, the level before transfusion. There was no hematuria or transfusion reaction. The transfusion blood apparently was eliminated within 2 days, while the patient's own blood was unaffected. The only objection to accepting these data is that at the time the work was done I did not realize the possibility of a serum becoming non-specifically agglutinating and I did not check the Group IV serum used with this point in view. When in a third case this same elimination appeared to be taking place it proved to be the fault of the serum. On going back over the work I find that in each case on one of the days on which these counts were made, counts were made on other patients which gave the expected number of corpuscles, but I am not sure that the same serum was used. Since I have checked this factor it is true that I have not had a case in which rapid elimination of Group IV corpuscles occurred, but it is also true that with one exception I have not had an opportunity to study patients who were so near the terminal stage.

Case 49.—The patient was a man, aged 68 years. He had had previous relapses. There was no free hydrochloric acid in the stomach. The red blood count was 1,930,000, hemoglobin 40 per cent, giving a color index of 1. The white blood count was 2,400. The diagnosis was pernicious anemia.

Case 64.—The patient was a woman, aged 36 years. She was very anemic and thin. For 18 months she had been growing gradually weaker, and was in an exhausted condition at the time of examination. There was evidence of combined sclerosis. The hemoglobin was 22 per cent, the red blood count 1,040,000, color index 1.0+, and leucocyte count 1,900. The diagnosis was pernicious anemia.

DISCUSSION.

This study of pernicious anemia patients during a series of transfusions seems to indicate that although in some cases at certain times there is much blood elimination, on the whole, blood destruction is quiescent. Of course, it must be recognized that in this estimate the factor of production is an unknown quantity. Group IV transfused corpuscles are, as a rule, not eliminated so quickly as they are in transfused persons without blood disease, so that so far as Group

IV corpuscles are concerned there is no abnormal blood elimination. The fact that Group IV corpuscles are not eliminated rules out the activity of any corpuscle poison during these periods. For in order to assume the activity of such a poison we will have to make it one that is not able to attack Group IV corpuscles, in which case the incidence of pernicious anemia should at least be lower among Group IV individuals than among others. In the 189 pernicious anemia patients that have presented themselves at this Clinic during this study 52 per cent were in Group IV, which happens to be a somewhat higher incidence than the occurrence of Group IV individuals found in general among our patients and donors. It must be that at the time when the patients were under observation there was no hemotoxin active. It has been argued that transfusion introduces something which neutralizes the hemotoxin. It does not seem probable that there would be a complete neutralization of the toxin and that the patient's symptoms would still persist. Although in some instances a remission was brought about by the transfusion, in most cases the patients returned for further transfusions after 3 months. 3 months, it will be remembered, is the average length of time that what were presumably the youngest Group IV transfused corpuscles lasted in four pernicious anemia patients who were under observation to the end of elimination, and it is probably also the length of life of the like group transfusions. As in a few instances Group IV transfused blood stayed while the patient's total blood count dropped greatly, it would not seem that the protection of the Group IV corpuscles was due to an antibody to this hypothetical hemotoxin introduced by the transfused blood.

In the two cases, Nos. 75 and 68, in which there was the most marked elimination, the Group IV transfusion was maintained and both the patients' corpuscles and the transfused like group corpuscles were eliminated. Since normal like corpuscles were eliminated, the elimination in these cases cannot be assumed to be due to any intrinsic weakness of the corpuscle. Since in this elimination there was a differentiation between like group corpuscles and Group IV corpuscles it would seem that the factor bringing it about must have been the result of an activity of the body itself. If we accept the two cases in which the transfused blood disappeared rapidly.

we have additional data that would seem to bear out this conclusion. In the case in which there were time data, the normal transfused blood disappeared in 2 days, while the count of the patient's blood was maintained. Here again there would seem to be a differentiation between groups which would seem to be attributable only to a physiologic blood-destroying function.

This speeding up of the blood-destroying activity of the body, which seems to be the basis of the abnormal periods of blood destruction which are seen in transfused pernicious anemia patients, might be due, it is conceivable, to the indirect effect of a present toxin, or it might be due to some past injury which makes it difficult for the organism to maintain a sufficient supply of some substance necessary to colloidal equilibrium of the corpuscles. The recent work of Clark and Evans showing a reduction in the hemolytic inhibitory powers of serum of pernicious anemia patients to a point markedly below what is found in normal serum, and my own finding that at least as far as transfused blood is concerned physiologic elimination is brought about by a spasmodic effort on the part of the organism, are suggestive with regard to the mechanism by which these sudden excessive eliminations seen in pernicious anemia are brought about.

SUMMARY AND CONCLUSIONS.

Evidence is presented to show that there is no hemolytic toxin producing the anemia in pernicious anemia.

Partial evidence is presented to show that the periods of active blood destruction which are seen as the exception in pernicious anemia cases during a series of transfusions are due to the activity of the blood-destroying organs of the body rather than to the intrinsic weakness of the pernicious anemia blood corpuscle.

It is questionable whether blood destruction is as important a factor in producing the anemia of pernicious anemia as it is at present usually assumed to be.

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